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ORIGINAL REPORT

Risk of Subsequent Malignant Neoplasms in Long-Term Hereditary Retinoblastoma Survivors After Chemotherapy and Radiotherapy

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ABSTRACT

Purpose
Hereditary retinoblastoma (Rb) survivors have increased risk of subsequent malignant neoplasms (SMNs). Previous studies reported elevated radiotherapy (RT) -related SMN risks, but less is known about chemotherapy-related risks.

Patients and Methods
In a long-term follow-up study of 906 5-year hereditary Rb survivors diagnosed from 1914 to 1996 and observed through 2009, treatment-related SMN risks were quantified using cumulative incidence analyses and multivariable Cox proportional hazards regression models with age as the underlying time scale.

Results
Nearly 90% of Rb survivors were treated with RT, and almost 40% received alkylating agent (AA)–containing chemotherapy (predominantly triethylenemelamine). Median follow-up time to first SMN diagnosis was 26.3 years. Overall SMN risk was not significantly elevated among survivors receiving AA plus RT versus RT without chemotherapy (hazard ratio [HR], 1.27; 95% CI, 0.99 to 1.63). AA-related risks were significantly increased for subsequent bone tumors (HR, 1.60; 95% CI, 1.03 to 2.49) and leiomyosarcoma (HR, 2.67; 95% CI, 1.22 to 5.85) but not for melanoma (HR, 0.74; 95% CI, 0.36 to 1.55) or epithelial tumors (HR, 0.89; 95% CI, 0.48 to 1.64). Leiomyosarcoma risk was significantly increased for survivors who received AAs at age < 1 (HR, 5.17; 95% CI, 1.76 to 15.17) but not for those receiving AAs at age ≥ 1 year (HR, 1.75; 95% CI, 0.68 to 4.51). Development of leiomyosarcoma was significantly more common after AA plus RT versus RT (5.8% *v* 1.6% at age 40 years; $P = .01$).

Conclusion
This comprehensive quantification of SMN risk after chemotherapy and RT among hereditary Rb survivors also demonstrates an AA-related contribution to risk. Although triethylenemelamine is no longer prescribed, our findings warrant further follow-up to investigate potential SMN risks associated with current chemotherapies used for Rb.

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INTRODUCTION

Retinoblastoma (Rb), the most common primary intraocular malignancy among children, is attributed to mutations in the retinoblastoma gene (*RB1*). Patients with hereditary Rb with bilateral (tumors present in both eyes) or unilateral disease (tumors in one eye) and a known family history of Rb are presumed to have a germline *RB1* mutation.[1] Advances in Rb treatment have contributed to improvements in survival, from a 3-year survival rate of 76% in the 1970s to a 5-year survival rate of 97% since the mid 1990s.[2,3] Survivors of hereditary Rb have an increased risk for developing a subsequent malignant neoplasm (SMN), most commonly bone, soft tissue sarcoma, or melanoma, relative to survivors of nonhereditary Rb and to the general population.[4-8] Previous studies among patients with Rb have reported elevated SMN risks associated with past use of radiotherapy (RT),[9-12] particularly when administered before age 1 year.[11,12] An increased incidence of common epithelial cancers, such as bladder, lung, and breast, has also been observed among Rb survivors.[13,14]

Rb treatment has changed considerably over the last several decades. Historically, treatment of an eye with advanced intraocular Rb without enucleation was irradiation, with most patients receiving

external-beam RT and a small number of patients receiving brachytherapy.[15] However, alternative therapeutic approaches were pursued because of the substantial adverse effects of RT, including irradiation vascular necrosis and hemorrhages, functional loss of the eye, and irradiation-induced SMNs.[15] In the 1950s, triethylenemelamine (TEM), an alkylating agent (AA) with high specificity for retinal cells, was introduced as adjuvant chemotherapy (CT) with the hope that combining it with irradiation would allow radiation doses to be lowered.[16,17] Case reports of SMNs among patients with Rb who had been treated with TEM and other agents[18-22] contributed to increased awareness of the potential adverse effects of these treatments and to the development of new chemotherapeutic approaches for these patients.[23-27]

Previous analyses of our ongoing study of Rb survivors have suggested an increased risk for SMNs among those treated with CT relative to survivors not treated with CT.[4,5] Analyses in other cohorts of childhood cancer survivors without Rb have also suggested an overall increased risk for SMNs associated with CT.[20,28-30] However, no previous study has formally quantified CT-related risks after Rb by SMN type or CT agent. Understanding potential late adverse effects of CT and RT will provide valuable clinical information regarding treatment and long-term follow-up of patients with Rb. Therefore, we quantified SMN risk after CT and RT in a long-term follow-up study of hereditary Rb survivors.

PATIENTS AND METHODS

Study Population

Study participants were identified from a retrospective cohort of Rb survivors, described previously in detail.[31] Briefly, 1,991 Rb survivors were diagnosed at major medical centers in New York, New York (from January 1, 1914, to December 31, 1996), and Boston, Massachusetts (from January 1, 1914, to December 31, 1984). Medical record abstraction from 1984 to 1985 and 1996 to 1997 collected retrospective data on treatments received for Rb, laterality, family history of Rb, SMN occurrence, and cause of death. We classified individuals with bilateral disease or those with unilateral disease and positive family history (excluding children) as hereditary.

This analysis was restricted to hereditary Rb survivors (N = 1,069). We excluded additional survivors for whom treatment was unknown (n = 28) or who survived < 5 years after Rb diagnosis (n = 135), because treatment-related SMNs were unlikely to occur before 5 years. These exclusions resulted in a final analytic population of 906 hereditary Rb survivors. We also excluded nonhereditary Rb survivors (n = 781) because of few reported SMNs (n = 29, of which 21 occurred after surgery only). The Special Studies Institutional Review Board of the National Cancer Institute as well as the institutional review boards of the two participating medical centers approved the study.

Rb Treatment Data

Hereditary Rb survivors in this analysis who received RT (n = 813) were treated with external-beam RT (85%), brachytherapy (11%), external-beam RT plus brachytherapy (3%), or an unspecified type of RT (1%). CT data included agents received and treatment dates for primary and recurrent Rb. Most survivors who received CT were treated within 5 years of Rb diagnosis (n = 340; 97%). For survivors who received CT > 5 years after Rb diagnosis (n = 9; 3%), CT was considered a time-dependent variable.

Most of the hereditary Rb survivors who received CT were treated with TEM (67%). An additional 28% of survivors were treated with a range of other AAs, including nitrogen mustard (2%), cyclophosphamide (23%), and thiotepa (6%). Only seven patients received non–AA-containing CT. Our main analyses compared Rb survivors who received any AA and RT (AA plus RT) with those who received RT without any CT. Secondary analyses stratified patients who received AAs into those who received TEM only versus those who received other AAs (with or without TEM). Investigation of other specific AAs was not possible, because of the small number of patients who received these agents.

SMN Ascertainment

Most SMNs were ascertained through periodic questionnaires. Response rates for the most recent questionnaires in 2000 and 2008 were 75% and 72%, respectively. Vital status and cause of death were obtained from the National Death Index. Reported SMNs were confirmed via pathology reports (47%), physician or hospital records (22%), autopsy reports (2%), or death certificates (29%).

All SMNs were classified according to the International Classification of Diseases for Oncology (third edition).[32] We evaluated risk of any SMN as well as the first occurrence of a specific type of SMN in at least 30 patients: bone tumors, soft tissue sarcoma, melanoma, and epithelial tumors. We further stratified soft tissue sarcomas by tissue of origin into leiomyosarcoma and other/unspecified soft tissue sarcoma.

Statistical Analysis

For each SMN subtype, follow-up began 5 years after Rb diagnosis and ended on the date of SMN diagnosis, death, or last completed questionnaire, whichever occurred earliest. To compare SMN risk with that expected in the general population, we estimated standardized incidence ratios (SIRs) and 95% CIs. SIRs among survivors treated with CT plus RT were compared with those among survivors treated with RT using the χ^2 test of homogeneity.[33] We also computed excess absolute risk ([O-E] × 10,000/person-years at risk). Expected numbers of patient cases were derived from age-, sex-, and calendar year–specific incidence rates from the Connecticut Tumor Registry (1935 to 1972) and SEER program (1973 to 2009).

We used Cox proportional hazards regression modeling with age as the time scale to examine SMN risk among survivors treated with CT plus RT, as well as the CT subgroups, relative to those treated with RT (without CT). Models were adjusted for sex, age at Rb diagnosis (< 1 $\nu \geq 1$ years). Because SMN treatments were not collected but could affect the risk of another malignancy, we included a time-dependent indicator variable for the development of SMNs other than the subtype of interest. Models were stratified by calendar year of diagnosis to account for temporal changes in treatment practices for Rb; approximately 95% of survivors treated with TEM plus RT were diagnosed before 1970, whereas 65% of survivors treated with other AAs were predominantly diagnosed after 1970. Furthermore, we tested whether there was an additional effect on SMN risk when an AA was received at age < 1 year by including an indicator variable. Analyses were conducted using SAS software (version 9.3; SAS Institute, Cary, NC). Two-sided P values < .05 were considered statistically significant.

Cumulative incidence for each SMN subtype was calculated and compared by treatment received, with death and loss to follow-up as competing risks. Analyses were conducted using Gray's cumulative incidence method implemented in the cmprsk package in R statistical software (http://www.r-project.org).[34]

RESULTS

A majority (86%) of the 906 survivors were diagnosed age < 2 years, and 95% were diagnosed with bilateral Rb (Table 1). Nearly 90% of survivors were treated with RT, and almost 40% received CT. Among the CT subgroups, most survivors were treated with TEM only (64%) or another AA with or without TEM (31%).

Among the 813 survivors treated with CT plus RT or RT, 265 (33%) developed at least one SMN (median follow-up, 26.3 years; range, 0.6 to 63.0 years), and 46 (6%) developed more than one SMN. For the main SMN subtypes of interest, 97% of the 92 bone tumors, 90% of the 92 soft tissue sarcomas, and 87% of the 31 melanomas were the survivor's first reported SMN. Median age at diagnosis of a bone tumor was 15.7 years, followed by melanoma at 30.2 years and soft tissue sarcoma at 31.6 years.

Table 1. Demographic and Clinical Characteristics of 5-Year Survivors of Hereditary Rb With Known Treatment (n = 906)

Characteristic	Surgery Only (n = 80)* No.	%	RT (n = 477) No.	%	CT Plus RT (n = 336) No.	%	CT (n = 13) No.	%
Sex								
Male	43	54	246	52	182	54	8	62
Female	37	46	231	48	154	46	5	38
Age at diagnosis, years								
< 1	32	40	314	66	188	56	6	46
1	26	33	115	24	94	28	4	31
2	13	16	35	7	42	13	3	23
≥ 3	9	11	13	3	12	4	0	0
Calendar year of diagnosis								
1914-1959	33	41	100	21	114	34	2	15
1960-1969	10	13	120	25	132	39	8	62
1970-1979	18	23	127	27	63	19	3	23
1980-1996	19	24	130	27	27	8	0	0
Laterality								
Unilateral	12	15	15	3	4	1	1	8
Bilateral	68	85	462	97	332	99	12	92
Family history								
No	56	70	334	70	261	78	9	69
Yes	19	24	115	24	62	18	4	31
Unknown	5	6	28	6	13	4	0	0
Enucleation								
None	4	5	99	21	16	5	0	0
Both eyes	23	29	70	15	141	42	6	46
One eye	53	66	306	64	179	53	7	54
Unknown	0	0	2	0	0	0	0	0
CT for Rb								
Non-AA	0	0	0	0	7	2	0	0
TEM only	0	0	0	0	216	64	2	15
AA (with or without TEM)†	0	0	0	0	104	31	11	85
Not ascertained	0	0	0	0	9	3	0	0
Age at last follow-up, years‡								
< 10	2	3	27	6	28	8	0	0
10-19	14	18	100	21	61	18	2	15
20-29	17	21	125	26	58	17	0	0
30-39	15	19	113	24	79	24	8	62
40-49	15	19	75	16	68	20	1	8
≥ 50	17	21	37	8	42	13	2	15
Vital status at last follow-up								
Alive	62	78	348	73	204	61	12	92
No SMN	58	73	324	68	173	51	12	92
SMN	4	5	24	5	31	9	0	0
Dead	18	23	129	27	132	39	1	8
No SMN	2	3	18	4	33	10	1	8
SMN	16	20	111	23	99	29	0	0

Abbreviations: AA, alkylating agent; CT, chemotherapy; Rb, retinoblastoma; RT, radiotherapy; SMN, subsequent malignant neoplasm; TEM, triethylenemelamine.

*Received combination of enucleation, cryotherapy, photocoagulation, or other surgical treatment.

†Other AAs received included carboplatin, chlorambucil, cisplatin, cyclophosphamide, melphalan, mitomycin, nitrogen mustard, and thiotepa.

‡Last follow-up defined as earliest occurrence of last completed questionnaire or death.

Compared with the expected risks in the general population, SMN risk was significantly elevated among hereditary Rb survivors treated with CT plus RT (n = 130; SIR, 26.4; 95% CI, 22.0 to 31.3) as well as those treated with RT (n = 135; SIR, 20.4; 95% CI, 17.1 to 24.2; Table 2). There was an excess of 157 and 119 patient cases per 10,000 persons per year among survivors who received CT plus RT and RT, respectively. By SMN subtype, SIRs among survivors treated with CT plus RT were significantly greater than those among survivors treated with RT for bone cancers (SIR, 676.9 *v* 422.1; $P = .03$) and leiomyosarcomas (SIR, 907.4 *v* 307.2; $P < .001$). In contrast, SIRs in the two treatment groups did not differ significantly for other/unspecified soft tissue sarcoma, melanoma, or epithelial tumors. SIRs stratified by follow-up time (< 25 *v* ≥ 25 years) demonstrated similar results (Appendix Table A1, online only).

Overall, treatment with CT plus RT was associated with a significantly increased risk of any SMN (hazard ratio [HR], 1.31; 95% CI, 1.02 to 1.68) compared with RT and no CT, with increased risks also observed for specific type of CT (Table 3). CT plus RT was also associated with increased risk of bone tumors (HR, 1.73; 95% CI, 1.13 to 2.67) but not soft tissue sarcomas (HR, 1.29; 95% CI, 0.84 to 1.97), melanomas (HR, 0.72; 95% CI, 0.35 to 1.50), or epithelial tumors (HR, 0.94; 95% CI, 0.51 to 1.73). Additional analysis by soft tissue sarcoma subtype demonstrated significantly increased risks for leiomyosarcomas (HR, 2.61; 95% CI, 1.19 to 5.70) among survivors who received CT plus RT but no differences by specific CT type. In analyses by tumor location, results were similar to overall risk estimates, except for a higher risk for bone tumors outside of the irradiated field among those who received CT plus RT relative to those who received RT (HR, 2.28; 95% CI, 1.02 to 5.11; Appendix Table A2, online only).

In exploratory analyses by age at receipt of AA, we observed similar risk estimates for any SMN when an AA was administered at age < 1 (HR, 1.40; 95% CI, 1.01 to 1.96) versus ≥ 1 year (HR, 1.17; 95% CI, 0.86 to 1.61; $P = .39$). In analyses by SMN subtype, the association with leiomyosarcoma was particularly pronounced for receipt of AAs at age < 1 (HR, 5.17; 95% CI, 1.76 to 15.17) but not ≥ 1 year (HR, 1.75; 95% CI, 0.68 to 4.51), although this difference by age was not statistically significant ($P = .08$). Risks of other/unspecified soft tissue sarcomas, bone tumors, melanoma, and epithelial tumors were similar regardless of age at AA receipt. Only the cumulative incidence of leiomyosarcomas was significantly higher for Rb survivors who received AA plus RT versus RT (5.8% and 1.6%, respectively, at age 40 years; $P = .01$; Fig 1; Appendix Table A3, online only).

DISCUSSION

Consistent with previous reports, we demonstrate an elevated risk for subsequent bone tumors, soft tissue sarcomas, and melanomas among long-term Rb survivors.[4-10] For the first time, to our knowledge, we provide evidence that the elevations in risk for bone tumors and leiomyosarcomas are higher for survivors who were treated with AA plus RT versus those who received RT without CT, whereas use of AA plus RT was not associated with elevated risk for melanoma. Our findings should heighten awareness of the potential CT-related risks for SMNs in the long-term management of Rb survivors.

Although previous studies have consistently demonstrated increased risks for bone cancers and soft tissue sarcomas among Rb survivors who received RT, investigations of CT and SMN development have been limited. Previous reports among hereditary Rb survivors in our study have suggested increased risks of bone cancers and soft tissue sarcomas associated with CT,[4,5] but our analysis represents, to our knowledge, the first comprehensive analysis of CT-related SMN

Table 2. SIRs for SMNs by Treatment Received Among 5-Year Survivors of Hereditary Rb

Outcome	RT*				CT Plus RT†				
	Observed	SIR	95% CI	EAR‡	Observed	SIR	95% CI	EAR‡	*P*
All SMNs§	135	20.4	17.1 to 24.2	119.2	130	26.4	22.0 to 31.3	156.7	.04
Bone tumor	44	422.1	306.7 to 566.7	38.8	48	676.9	499.1 to 897.5	56.9	.03
Soft tissue sarcoma	46	123.5	90.4 to 164.7	40.6	46	145.1	106.2 to 193.5	54.6	.49
Leiomyosarcoma‖	10	307.2	147.3 to 564.9	8.7	22	907.4	568.7 to 1,373.9	25.9	< .001
Other/unspecified soft tissue sarcoma	36	104.4	73.1 to 144.5	31.6	24	79.1	50.7 to 117.6	27.9	.29
Melanoma	19	32.3	19.5 to 50.5	16.2	12	23.8	12.3 to 41.6	13.6	.42
Epithelial tumor¶	25	6.2	4.0 to 9.2	18.5	21	6.8	4.2 to 10.3	21.0	.77

NOTE. Survivors who underwent surgery (n = 80) or received CT only (n = 13) were excluded, because few or no subsequent neoplasms were reported in these treatment groups.

Abbreviations: CT, chemotherapy; EAR, excess absolute risk; Rb, retinoblastoma; RT, radiotherapy; SIR, standardized incidence ratio; SMN, subsequent malignant neoplasm.

*Total of 477 survivors and 10,769.9 person-years at risk.

†Total of 336 survivors and 7,981.9 person-years at risk.

‡Per 10,000 persons.

§First SMNs included bone tumors (n = 89), soft tissue sarcomas (n = 81), melanomas (n = 27), and epithelial tumors (n = 40).

‖Leiomyosarcomas were diagnosed in abdomen (n = 1), cecum (n = 2), head, face, or neck (n = 1), lower limb or hip (n = 2), pelvis (n = 2), maxillary sinus (n = 3), nasal cavity (n = 2), nasopharynx (n = 1), orbit (n = 4), retroperitoneum (n = 2), scrotum (n = 1), sphenoid sinus (n = 1), trunk (n = 1), upper limb or shoulder (n = 2), uterus (n = 6), and unknown site (n = 1).

¶Epithelial cancers were diagnosed in bladder (n = 5), breast (n = 8), colorectum (n = 1), kidney (n = 2), lung (n = 6), nasal cavity (n = 12), prostate (n = 1), retroperitoneum (n = 2), thyroid (n = 3), tongue (n = 2), and uterus (n = 4).

risk. In addition, a study of 46 survivors who received TEM and RT reported seven SMNs, including sarcomas of the femur and orbit, as well as other cancers occurring in the brain, pineal gland, parotid gland, and cervix.[21] Among the 18 Rb survivors who developed a subsequent osteosarcoma in the Late Effects Study Group, seven and six survivors had received TEM or cyclophosphamide, respectively.[22] Osteosarcoma was also reported among Rb survivors after receiving cyclophosphamide in two other studies.[19,20] Of 25 survivors who received RT and developed subsequent soft tissue or bone sarcomas in another study, almost half had received cyclophosphamide, either alone or in combination with other agents.[35] Individual cases of bone cancers have also been reported in patients who received TEM.[18] Although one study did report few SMNs after Rb treatment with carboplatin, vincristine, and etoposide, mean follow-up time for hereditary survivors was only 6.67 years.[36] Additional follow-up in this cohort is needed to capture the typical ages for SMN development. Furthermore, in a case series of 15 Rb survivors with secondary acute myelogenous leukemia, 12 had been treated with CT, including topoisomerase II inhibitors, epipodophyllotoxins, and AAs.[37]

Although studies of CT-related SMN risks among Rb survivors are limited, our results are consistent with previous studies reporting CT-related risks of bone cancers among childhood cancer survivors. Similar to our findings, a case-control study using the UK National Registry of Childhood Tumors reported a nonsignificant 2.1-fold increased risk for bone cancers in the CT plus RT group relative to the group receiving RT.[20] In another LESG case-control analysis, childhood cancer survivors who had an alkylator score ≥ 3 and had received $\geq 1{,}000$ rad had a 1.6-fold increased risk for bone cancers.[30] In both of those studies, a supra-additive effect was observed when comparing the CT plus RT relative risk with the independent risks for RT and CT, suggesting a greater risk when survivors are treated with both RT and CT as opposed to RT or CT alone.[20,30] Our study was limited by the small number of Rb survivors who were treated with CT but no RT and by no reported SMNs in this treatment group; thus, we were

Table 3. Risk for SMNs by Treatment Received Among 5-Year Survivors of Hereditary Rb (n = 813)

Outcome	RT*	CT Plus RT			AA Plus RT			TEM Plus RT			Other AA Plus RT		
	No.	No.	HR	95% CI	No.	HR	95% CI	No.	HR	95% CI	No.	HR	95% CI
All SMNs	135	130	**1.31**	**1.02 to 1.68**	124	1.27	0.99 to 1.63	101	1.27	0.96 to 1.68	23	1.18	0.77 to 1.80
Bone tumor	44	48	**1.73**	**1.13 to 2.67**	44	**1.60**	**1.03 to 2.49**	32	1.48	0.88 to 2.47	12	1.70	0.88 to 3.28
Soft tissue sarcoma	46	46	1.29	0.84 to 1.97	45	1.30	0.84 to 1.99	38	1.40	0.88 to 2.25	7	1.05	0.46 to 2.39
Leiomyosarcoma	10	22	**2.61**	**1.19 to 5.70**	22	**2.67**	**1.22 to 5.85**	20	**3.21**	**1.40 to 7.39**	2	1.65	0.31 to 8.80
Other/unspecified soft tissue sarcoma	36	24	0.89	0.52 to 1.52	23	0.87	0.51 to 1.51	18	0.85	0.46 to 1.57	5	0.94	0.36 to 2.44
Melanoma	19	12	0.72	0.35 to 1.50	12	0.74	0.36 to 1.55	11	0.83	0.38 to 1.78	1	—	—
Epithelial tumor	25	21	0.94	0.51 to 1.73	20	0.89	0.48 to 1.64	16	0.78	0.40 to 1.52	4	1.07	0.34 to 3.36

NOTE. Survivors who underwent surgery (n = 80) or received CT only (n = 13) were excluded, because few or no subsequent neoplasms were reported in these treatment groups. Adjusted for sex, age at retinoblastoma diagnosis (< 1 *y* ≥ 1 year), calendar year of Rb diagnosis (1914-1959, 1960-1969, 1970-1979, or 1980-1996), and time-dependent covariate for prior SMN diagnosis. Bold font indicates statistical significance at $P < .05$.

Abbreviations: AA, alkylating agent; CT, chemotherapy; HR, hazard ratio; Rb, retinoblastoma; RT, radiotherapy; SMN, subsequent malignant neoplasm; TEM, triethylenemelamine.

*Reference group for all HR calculations.

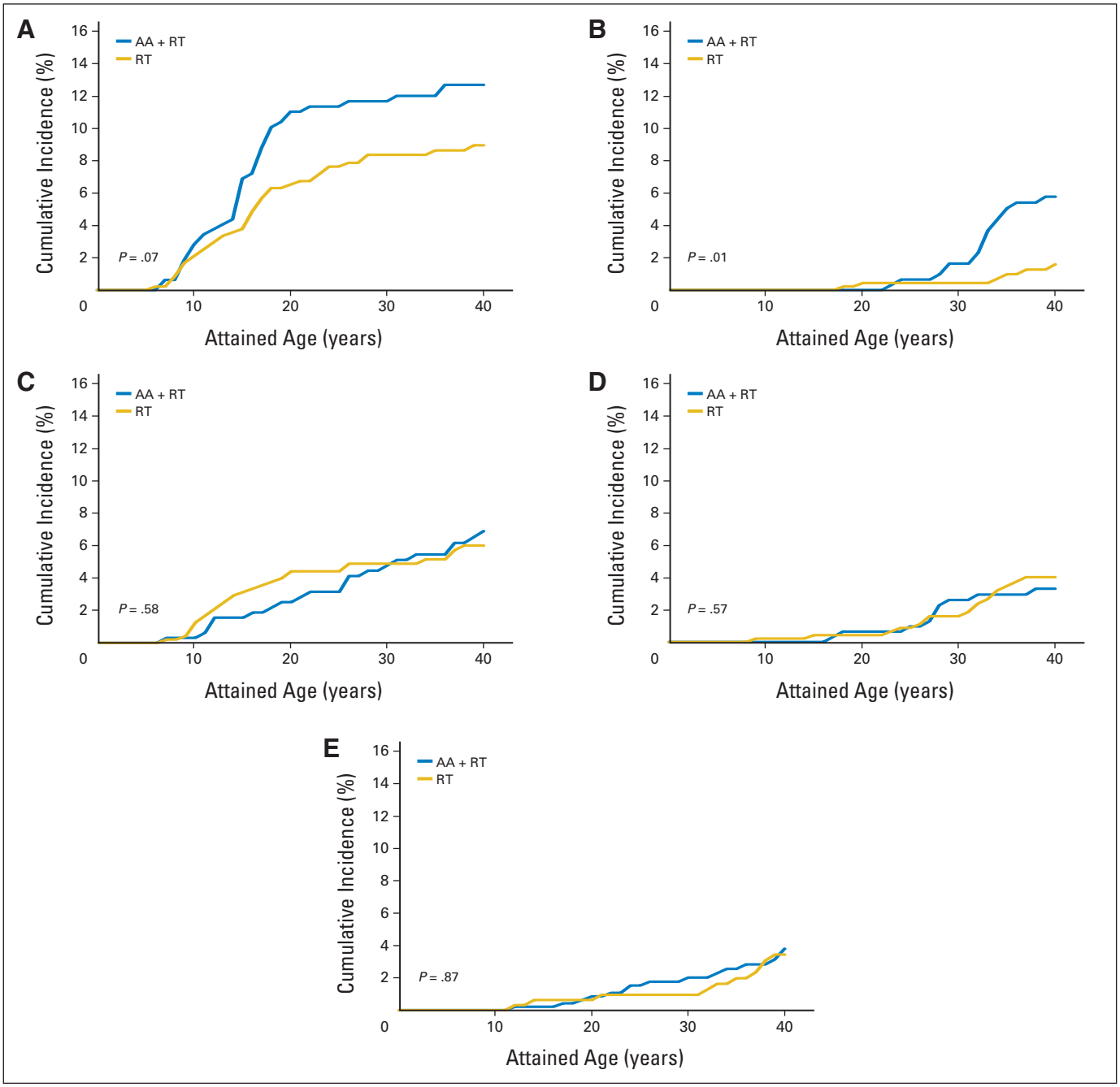

Fig 1. Cumulative incidence of subsequent malignant neoplasms after retinoblastoma by treatment received. (A) Bone tumor; (B) leiomyosarcoma; (C) other/unspecified soft tissue sarcoma; (D) melanoma; and (E) epithelial tumor. AA, alkylating agent; RT, radiotherapy.

unable to estimate SMN risks associated with CT alone. However, the previously mentioned LESG study also demonstrated a positive dose-response relationship for the alkylator score with bone sarcoma risk among childhood cancer survivors treated with CT only.[30]

We also observed an increased risk for soft tissue sarcomas, specifically leiomyosarcomas, among Rb survivors who received CT and RT. A case-control analysis of a United Kingdom–based cohort of childhood cancer survivors reported a positive dose-response relationship for soft tissue sarcoma among survivors treated with AAs.[28] However, we lacked adequate data on drug doses to evaluate a potential dose-response relationship. Previous studies among Rb survivors have reported higher SMN risk with RT administered before age 1 year.[11,12] Although the AA-related risk estimate for leiomyosarcomas was higher for receipt of AAs at age < 1 than for age ≥ 1 year, this difference was not statistically significant, and thus, it remains unclear whether treatment-related risks differ by age. Further research is needed to understand whether younger individuals may be more susceptible to AA-related SMNs. Other studies also noted a particularly elevated risk for leiomyosarcomas compared with other soft tissue sarcomas after Rb, but those studies lacked data on Rb

treatments.[10,14,38] Loss of heterozygosity in *RB1* and in other major tumor suppressor genes,[39-43] as well as deletions of chromosome 13, that contain the *RB1* gene[44] has been reported in individuals who developed uterine leiomyosarcomas. Future genetic studies in this population could elucidate the predisposition for leiomyosarcomas in patients with Rb.

In contrast to our findings for bone cancers and leiomyosarcomas, CT was not associated with melanoma risk after Rb, which is consistent with previous reports.[5,14] Development of melanoma may be related to an underlying genetic predisposition associated with Rb rather than treatment.[4] Major susceptibility genes for melanoma include *CDKN2A* and *CDK4*, which are both upstream from the *RB1* gene.[45] Additional investigation is necessary to understand the association between melanoma and Rb.

Whereas the leukemogenicity of certain chemotherapeutic agents is well established,[46] our findings add to a growing body of evidence for associations between AAs and a range of solid SMNs. CT has also been associated with an increased risk for lung cancer after Hodgkin and non-Hodgkin lymphomas,[47,48] stomach cancer after Hodgkin lymphoma (in combination with high-dose abdominal RT),[49] and colorectal cancer after childhood cancer.[50] Anthracyclines also have been associated with increased sarcoma risk after childhood cancer, especially after Hodgkin lymphoma or a primary sarcoma.[51]

Most survivors in our analysis who received an AA received TEM, which is no longer used in clinical practice. Current CT agents recommended for Rb include cyclophosphamide, ifosfamide, carboplatin, vincristine, etoposide, topotecan, and doxorubicin.[23-27,52] On the basis of the recently developed cyclophosphamide equivalent dose,[53] TEM has substantially lower hematologic toxicity than agents used in current clinical practice (Appendix Table A4, online only), although toxicity to other tissues is not clear. Because of the long latency period of SMN development and potentially different CT drug–related adverse effects, further study is warranted to evaluate SMN risk with long-term follow-up of patients with Rb treated with current agents.

Several limitations of our study should be taken into account. We relied on reports of family history of Rb and laterality to define hereditary status. Some unilateral Rb survivors could have had a germline *RB1* mutation and should have been included in our analysis. However, we anticipate this number to be small based on other studies.[54-58]

Although some survivors were lost to follow-up, SMN risk estimates were unlikely to be affected, because response was not related to treatment received for Rb.[59] Although SMNs ascertained from the National Death Index are more likely to be misclassified because histology is not specified, sensitivity analyses excluding these SMNs yielded results similar to those of our main analysis. Similarly, sensitivity analyses including 1-year (*v* 5-year) survivors also yielded comparable results. In addition, although the cumulative incidence function takes into account the hazards of all other causes, whereas the Cox model is focused on a cause-specific hazard regardless of other causes, our results were similar in both analyses.

Hereditary Rb survivors treated with AA plus RT have a significantly higher risk of developing bone cancers and leiomyosarcomas than those treated with RT. With a median age at Rb diagnosis of only 9.36 months, excess risks associated with AA plus RT persist for decades, as demonstrated by the significantly higher incidence of leiomyosarcomas diagnosed at a median age of 34.3 years. Clinicians should be aware of these risks during long-term follow-up of Rb survivors. Further investigation of CT-related SMN risk among Rb survivors, particularly in patients treated with CT without RT, will inform risk-benefit assessments for current treatments and guide recommendations for future treatment protocols.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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Appendix

Table A1. SIRs for SMNs by Treatment Received and Follow-Up Among 5-Year Survivors of Hereditary Rb (n = 813)

Outcome (years of follow-up)	RT* Observed	RT* SIR	RT* 95% CI	RT* EAR‡	CT Plus RT† Observed	CT Plus RT† SIR	CT Plus RT† 95% CI	CT Plus RT† EAR‡	*P*
All SMNs									
< 25	75	53.1	41.7 to 66.5	98.3	64	62.9	48.5 to 80.3	119.1	.33
≥ 25	60	11.6	8.8 to 14.9	166.8	66	16.9	13.0 to 21.5	230.4	**.03**
Bone tumor									
< 25	36	452.2	316.7 to 626.1	46.9	40	788.0	563.0 to 1,073.0	74.8	**.01**
≥ 25	8	324.9	140.3 to 640.1	21.7	8	397.0	171.4 to 782.3	25.9	.69
Soft tissue sarcoma									
< 25	25	218.3	141.3 to 322.3	32.5	13	165.2	87.9 to 282.4	23.9	.32
≥ 25	21	81.4	50.4 to 124.4	57.9	33	138.4	95.3 to 194.4	110.6	**.05**
Leiomyosarcoma									
< 25	2	391.5	47.4 to 1,414.2	2.6	2	513.9	62.2 to 1,856.5	3.7	.34
≥ 25	8	291.5	125.9 to 574.4	21.7	20	982.7	600.2 to 1,517.6	65.7	**< .001**
Other/unspecified soft tissue sarcoma									
< 25	23	209.9	133.0 to 314.9	29.9	11	146.5	73.1 to 262.2	20.2	.25
≥ 25	13	55.3	29.4 to 94.5	35.2	13	56.9	30.3 to 97.3	41.6	.91
Melanoma									
< 25	4	37.6	10.2 to 96.1	5.0	4	49.3	13.4 to 126.2	7.2	.65
≥ 25	15	31.2	17.4 to 51.4	40.1	8	18.9	8.2 to 37.3	25.1	.25
Epithelial tumor									
< 25	8	30.9	13.3 to 60.8	10.0	4	19.3	5.3 to 49.5	7.0	.43
≥ 25	17	4.5	2.6 to 7.2	37.0	17	5.9	3.4 to 9.4	45.8	.44

NOTE. Survivors who underwent surgery (n = 80) or received CT only (n = 13) were excluded, because few or no subsequent neoplasms were reported in these treatment groups. Bold font indicates statistical significance at $P < .05$.

Abbreviations: CT, chemotherapy; EAR, excess absolute risk; Rb, retinoblastoma; RT, radiotherapy; SIR, standardized incidence ratio; SMN, subsequent malignant neoplasm.

*Total of 477 survivors and 10,769.9 person-years at risk.

†Total of 336 survivors and 7,981.9 person-years at risk.

‡Per 10,000 persons.

Table A2. Risk for SMNs by Treatment Received and Tumor Location Among 5-Year Survivors of Hereditary Rb (n = 813)

Outcome	RT* No.	CT Plus RT No.	CT Plus RT HR	CT Plus RT 95% CI
Bone				
In field	25	27	1.44	0.82 to 2.52
Out of field	12	15	**2.28**	**1.02 to 5.11**
Leiomyosarcoma				
In field	4	8	2.50	0.73 to 8.58
Out of field	6	13	2.57	0.91 to 7.27
Other/unspecified soft tissue sarcoma				
In field	27	17	0.82	0.44 to 1.54
Out of field	3	3	1.50	0.28 to 7.99
Melanoma				
In field	5	4	0.87	0.23 to 3.26
Out of field	9	8	1.04	0.39 to 2.78
Epithelial tumor				
In field	11	7	0.59	0.20 to 1.79
Out of field	15	14	0.86	0.37 to 1.97

NOTE. Survivors who underwent surgery (n = 80) or received CT only (n = 13) were excluded, because few or no subsequent neoplasms were reported in these treatment groups. Adjusted for sex, age at retinoblastoma diagnosis (< 1 *v* ≥ 1 year), calendar year of Rb diagnosis (1914-1959, 1960-1969, 1970-1979, or 1980-1996), and time-dependent covariate for prior SMN diagnosis. Bold font indicates statistical significance at $P < .05$.

Abbreviations: CT, chemotherapy; HR, hazard ratio; Rb, retinoblastoma; RT, radiotherapy; SMN, subsequent malignant neoplasm.

*Reference group for all HR calculations.

Table A3. Cumulative Incidence of Subsequent Malignant Neoplasms After Retinoblastoma by Treatment Received

	Radiotherapy						Alkylating Agent With Radiotherapy						
	20 Years			40 Years			20 Years			40 Years			
Cancer Site	No.	Cumulative Incidence (%)	95% CI	No.	Cumulative Incidence (%)	95% CI	No.	Cumulative Incidence (%)	95% CI	No.	Cumulative Incidence (%)	95% CI	*P*
Bone	345	6.5	3.9 to 9.1	109	9.0	3.6 to 14.4	227	11.0	6.9 to 15.1	105	12.7	6.3 to 19.1	.07
Leiomyosarcoma	349	0.4	0.0 to 1.1	109	1.6	0.0 to 4.0	237	0.0	0.0 to 0.0	103	5.8	1.3 to 10.3	**.01**
Other/unspecified soft tissue sarcoma	343	4.4	2.2 to 6.6	109	6.0	1.5 to 10.5	235	2.5	0.5 to 4.5	103	6.9	2.0 to 11.8	.58
Melanoma	349	0.4	0.0 to 1.1	110	4.0	0.3 to 7.7	236	0.6	0.0 to 1.6	103	3.3	0.0 to 6.7	.57
Epithelial	347	0.8	0.0 to 1.7	108	3.8	0.2 to 7.4	237	0.6	0.0 to 1.6	105	3.4	0.0 to 6.9	.87

NOTE. Bold font indicates statistical significance at $P < .05$.

Table A4. CED Estimates for Typical Chemotherapies Received for Rb

Dose	Cyclophosphamide[53]	Ifosfamide[53]*	Carboplatin[53]†	Nitrogen Mustard[53]‡	TEM[17]§
Equivalent dose	100 mg/m^2	409 mg/m^2	29 mg/m^2	1 mg/m^2	0.3 mg/m^2
Equivalent dose factor‖	1.0	0.244	3.448	100	333
Typical dose for Rb	120 mg/m^2	1,600 mg/m^2 × 5 days or 3,000 mg/m^2 × 2 days	200 mg/m^2	12 mg/m^2	0.2 mg/m^2
CED¶	**120 mg/m^2**	**1,464 to 1,952 mg/m^2**	**689.6 mg/m^2**	**1,200 mg/m^2**	**66.6 mg/m^2**

NOTE. Bold font indicates statistical significance at $P < .05$.

Abbreviations: CED, cyclophosphamide equivalent dose; Rb, retinoblastoma; TEM, triethylenemelamine.

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‖CED/dose of drug of interest.

¶Equivalent dose factor × typical dose for Rb.